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Sensing Insulin Deterioration

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Abstract

Type-1 and type-2 diabetes is an affliction affecting 371 million people worldwide. To manage this disease, patients use insulin to lower their blood sugar levels. However, patients are unable to sense if their insulin has become deteriorated without sending the substance back to the manufacturer, adding costs to the manufacturer and patient. A spectrophotometer machine will evaluate insulin samples at specific wavelengths to demonstrate deterioration. A high performance liquid chromatography machine will then check these same insulin samples' potency. These machines will be utilized in parallel to determine if they can effectively determine if an insulin sample is deteriorated.

1. Introduction:

Insulin is a protein chain secreted from the pancreas' beta cells in healthy humans and lowers blood sugar levels. Presently, hundreds of millions of patients depend on insulin injections to survive, and insulin production has become a $17 billion dollar industry worldwide. Before the 1980s, insulin was harvested from bovine, pigs, and other animals. To meet the increasing diabetic population's insulin demands, insulin is now a product of recombinant DNA technologies, which are capable of large-scale production. Predominantly, E Coli is genetically modified to secrete insulin [1].

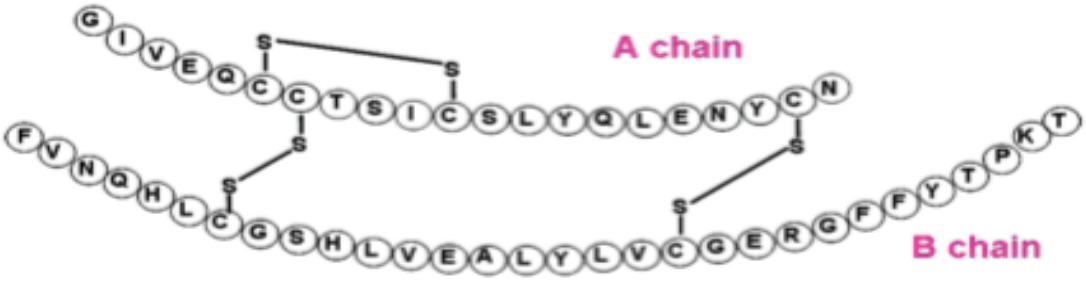

Figure 1: Amino acid sequence of insulin [2]

Diabetes is a disease that affects the ability of insulin to maintain blood sugar levels. For a patient with type-two diabetes, the patient becomes insulin resistant and may need additional insulin injections to lower blood sugar levels if the disease worsens. Juvenile diabetics', or type-one diabetics', own immune system will attack beta cells in the pancreas — beta cells store and secrete insulin — rendering the person with no self-synthesizing insulin system [3]. Due to this, type-one diabetics require insulin injections for life.

Insulin is well documented as having an inconsistent effect due to fragile physical stabilities [2]. After exposed to excessive heat, shaking, or expiration, insulin's ability to lower a diabetic's blood sugar levels can become inhibited or nonexistent. Damaged insulin can be dangerous for an unsuspecting diabetic, who will assume the insulin will lower his/her blood sugar levels. This could lead to high blood sugar, which can result in ketoacidosis, coma, or death. There is currently no technology and no reliable process available outside of a lab setting to determine if insulin has become damaged.

Literature suggests that damaged insulin has become unfolded and fibrillated. Fibrillation is the physical process by which partially unfolded insulin molecules interact with each other to form linear aggregates, which renders the insulin significantly weaker or ineffective for the patient [4]. Insulin's polypeptide unfolding and fibrillation can be detected qualitatively using a spectrophotometer at specific wavelengths. For a normal insulin sample, a relatively low absorbance reading will be detected around 275 nm. For a fibrillated insulin sample, a relatively large absorbance reading will be detected around 275 nm [5].

To determine the concentration of the insulin, high performance liquid chromatography (HPLC) is ideal. The HPLC method is quick (less than two hours per sample), cheap, and is easily automated for continuous analysis. The animal response methods are expensive and time-consuming. HPLC has been documented to give as accurate readings as animal response assays. In literature [6], a reversed-phase HPLC with a 75 x 4.6 mm i.d. column was packed with

Ultrapore RPSC at ambient temperature. Mobile phase A was 0.05% (v/v) trifluoroacetic acid in water. Mobile phase B was 0.05% trifluoroacetic acid in acetonitrile-water (60:40, v/v). The gradient was linear from 40% B to 44% B in 30 min. The flow rate was 2 ml min^{-1}, and detection was at 214 nm with a range setting of 0.2 a.u.f.s. Manufactured insulin peaked around 10 minutes in this study, while fibrillated insulin peaked around 20 minutes [6].

By completing this research, it is plausible for a MEMS (microelectromechanical system) spectrophotometer biosensor or MEMS HPLC biosensor to be created that will detect insulin fibrillation. If such a sensor is created, diabetics will have an at-home solution to tell if their insulin is at full strength.

2. Hypothesis:

We hypothesize that insulin deterioration detected by a spectrophotometer can be quantified by HPLC and is consistent for all major brands of insulin.

3. Procedures/Methods:

Samples of Humalog, Lantus, Novolog, and Levemir with concentrations of 100 units per milliliter were obtained. Each insulin was tested in a spectrophotometer at five dilutions, and the absorbance readings of the peaks were recorded. These same samples were then analyzed by an HPLC to check the potency of each sample, which created a standard curve. As shown in Figure 2, Novolog and Levemir have higher insulin concentrations than Lantus and Humalog. Therefore, the dilutions used for Novolog and Levemir were 20x, 40x, 80x, 160x, and 320x dilutions when constructing the standard curves, while Humalog and Lantus used 10x, 20x, 40x, 80x, and 160x dilutions. All heated samples were diluted to a 20x dilution.

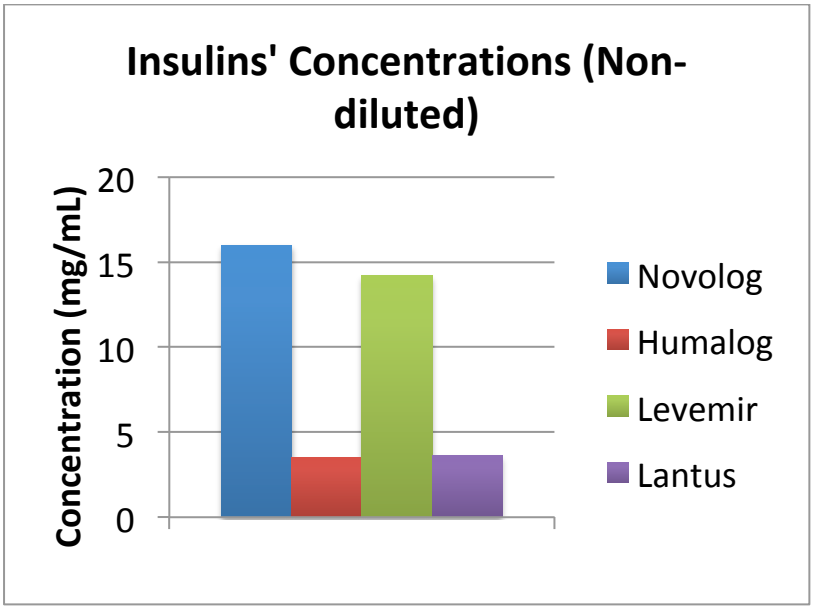

Figure 2: The relative concentrations of the insulins were used to determine the dilutions used for the standard curve constructions.

Three samples of each insulin type were then be placed into a hot water bath for 2 hours at 62 degrees Celsius. The heated samples were then tested in the spectrophotometer and HPLC. The spectrophotometer readings of the heated insulin samples were graphically compared to the normal insulin samples at 20x dilution. For the HPLC, mobile phase A composed of 0.05% (v/v) trifluoroacetic acid in water and mobile phase B composed of 0.05% trifluoroacetic acid in acetonitrile was used for Lantus. The three other insulins used mobile phase A composed of 0.1% formic acid in water and mobile phase B composed of 0.1% formic acid in acetonitrile. The flow rate was 1.3 ml min^{-1}, and the detection wavelength was at 280 nm for all insulins. From the linear standard curve created by the HPLC readings, the concentration of the heated insulins can be found using the HPLC readings. All samples are filtered before entering the HPLC.

4. Results:

The results of each insulin type will be discussed, which includes the standard curves, percent deterioration from heat, and spectrophotometer readings. For each insulins' standard curve equation, *y* is the area under the curve of the insulin peak, and *x* is the concentration of the insulin.

4.1 Lantus:

The standard curve for the Lantus insulin is shown in Figure 3.

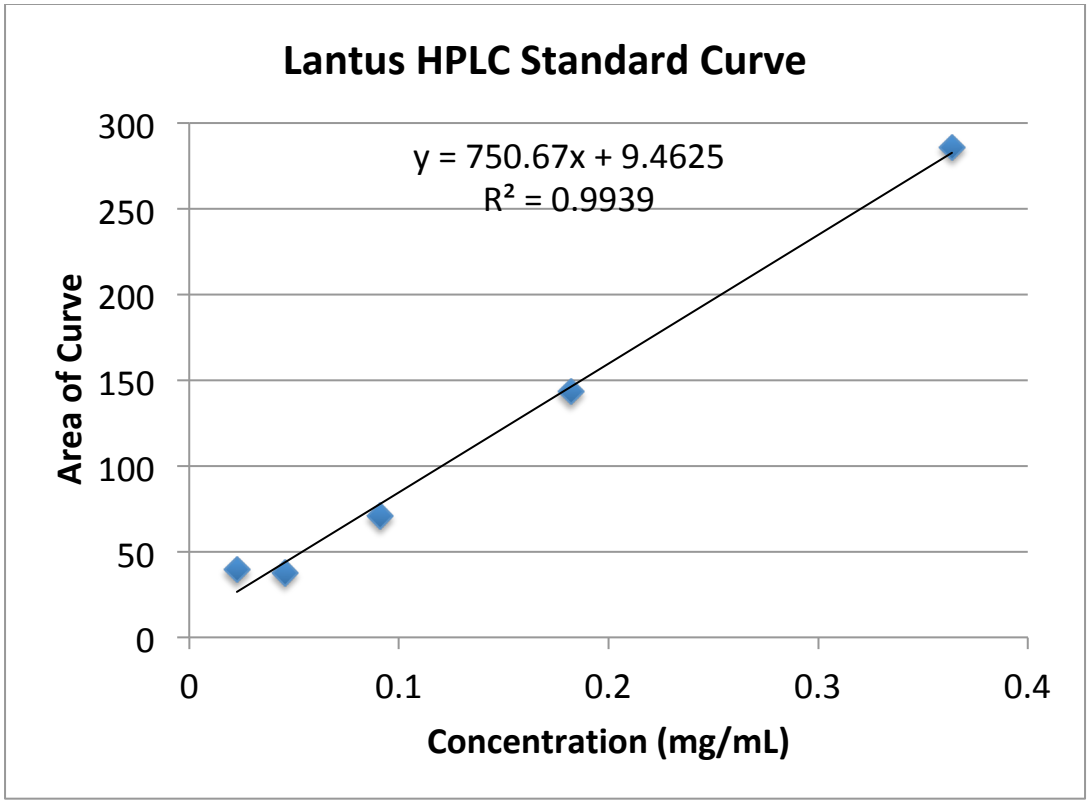

Figure 3: The standard curve will be used to find the incubated Lantus's concentration

From the three incubated samples, the average area is 83.9, which results in a concentration of 0.0991 mg/mL of insulin. This is a 45.5% reduction in the insulin concentration after heating. The spectrophotometer reading's for the 20x heated Lantus and the 20x un-heated Lantus are shown in Figure 4.

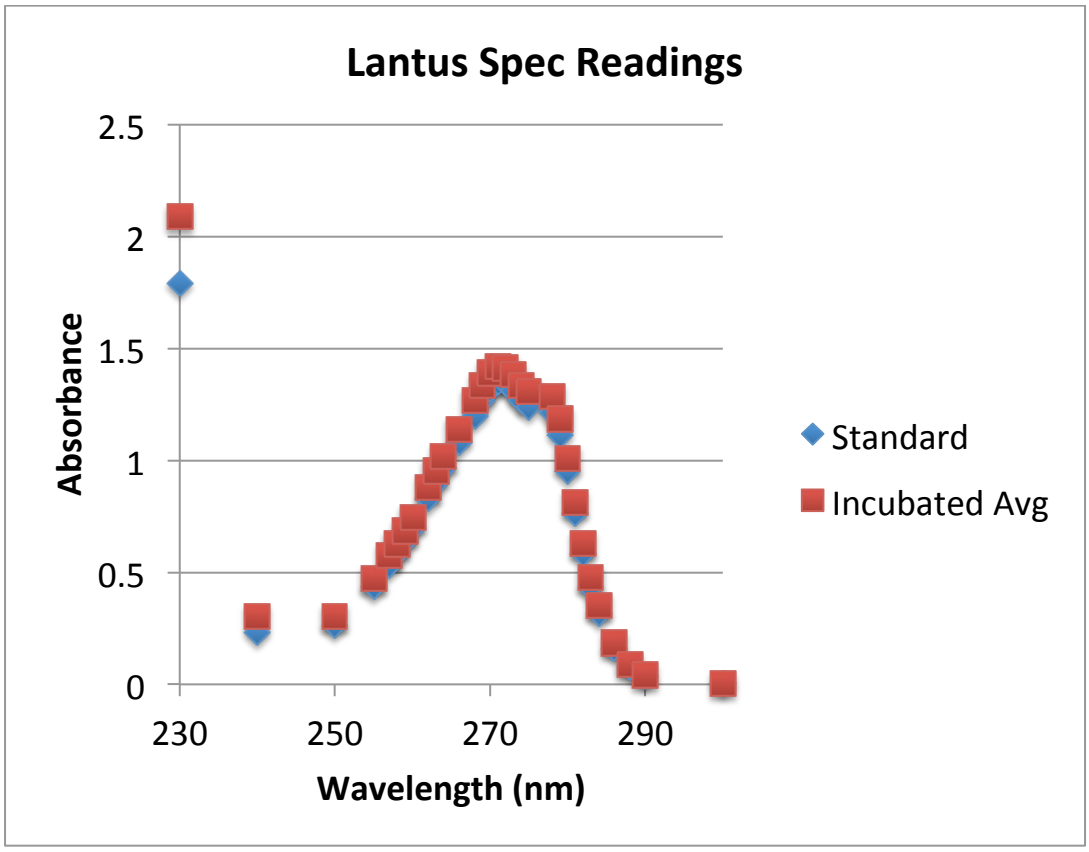

Figure 4: The incubated sample has a slightly higher peak than the normal sample.

4.2 Humalog:

The standard curve for the Humalog insulin is shown in Figure 5. Please note that the 80x dilution reading was omitted due to it being an outlier; it produced an area of 0.

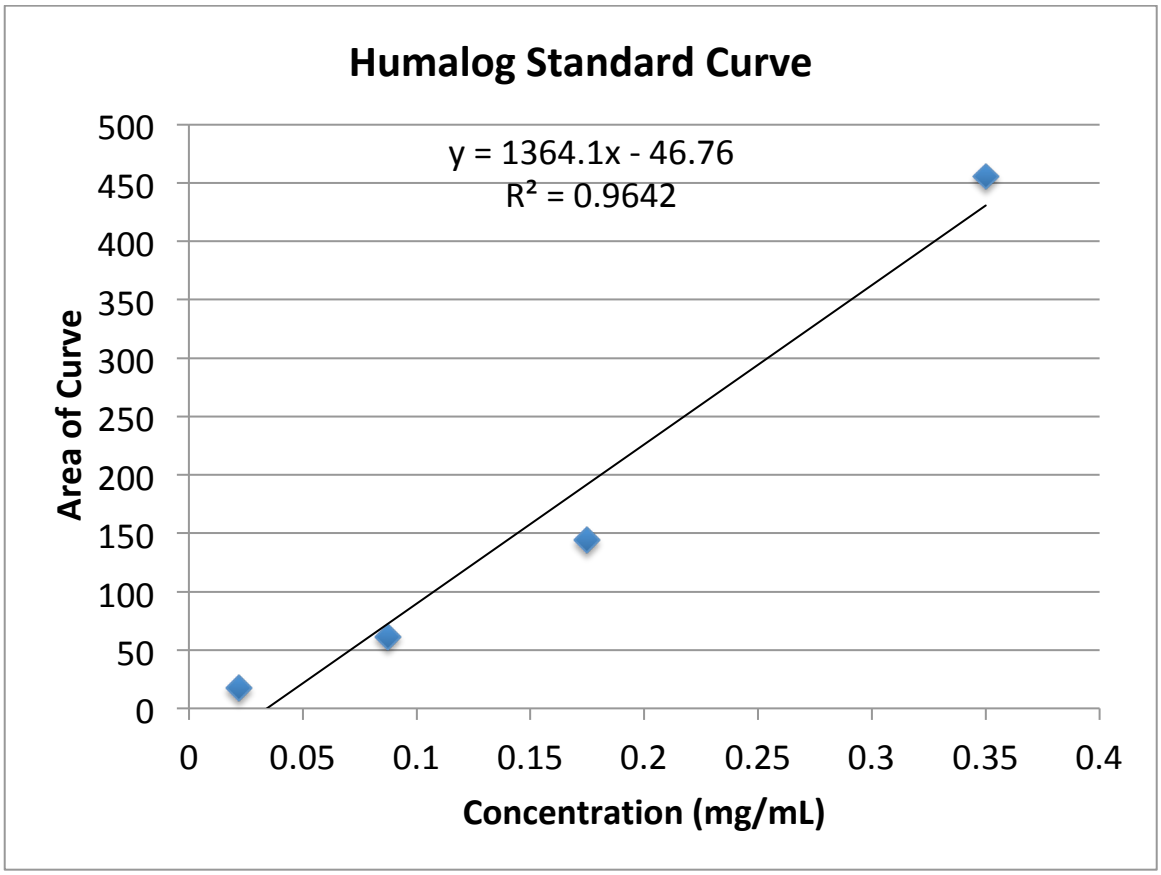

Figure 5: The standard curve will be used to find the incubated Humalog's concentration

From the three incubated samples, the average area is 87.0, which results in a concentration of 0.0980 mg/mL of insulin. This is a 44.0% reduction in the insulin concentration after heating. The spectrophotometer reading's for the 20x heated Humalog and the 20x un-heated Humalog are shown in Figure 6.

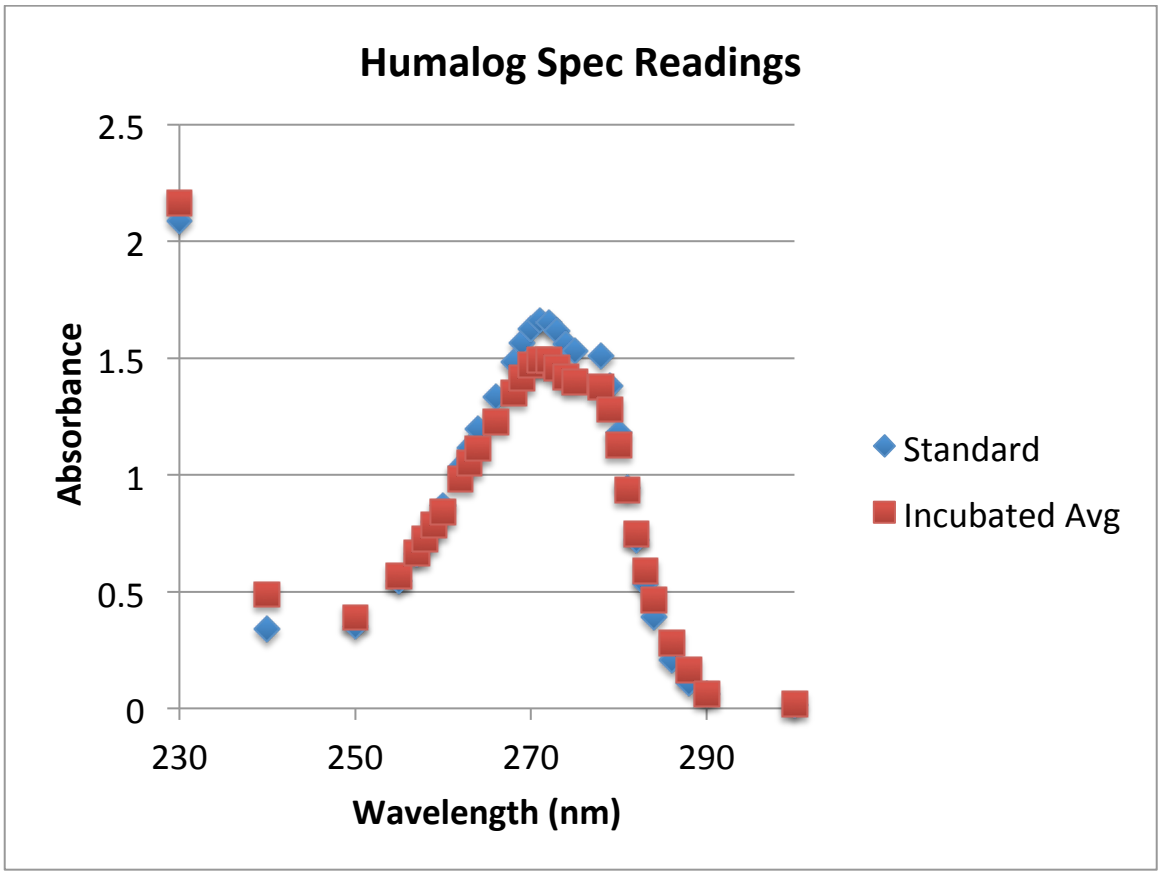

Figure 6: The incubated sample has a lower peak than the normal sample. This may be due to outliers in the data.

4.3 Levemir:

The standard curve for the Levemir insulin is shown in Figure 7.

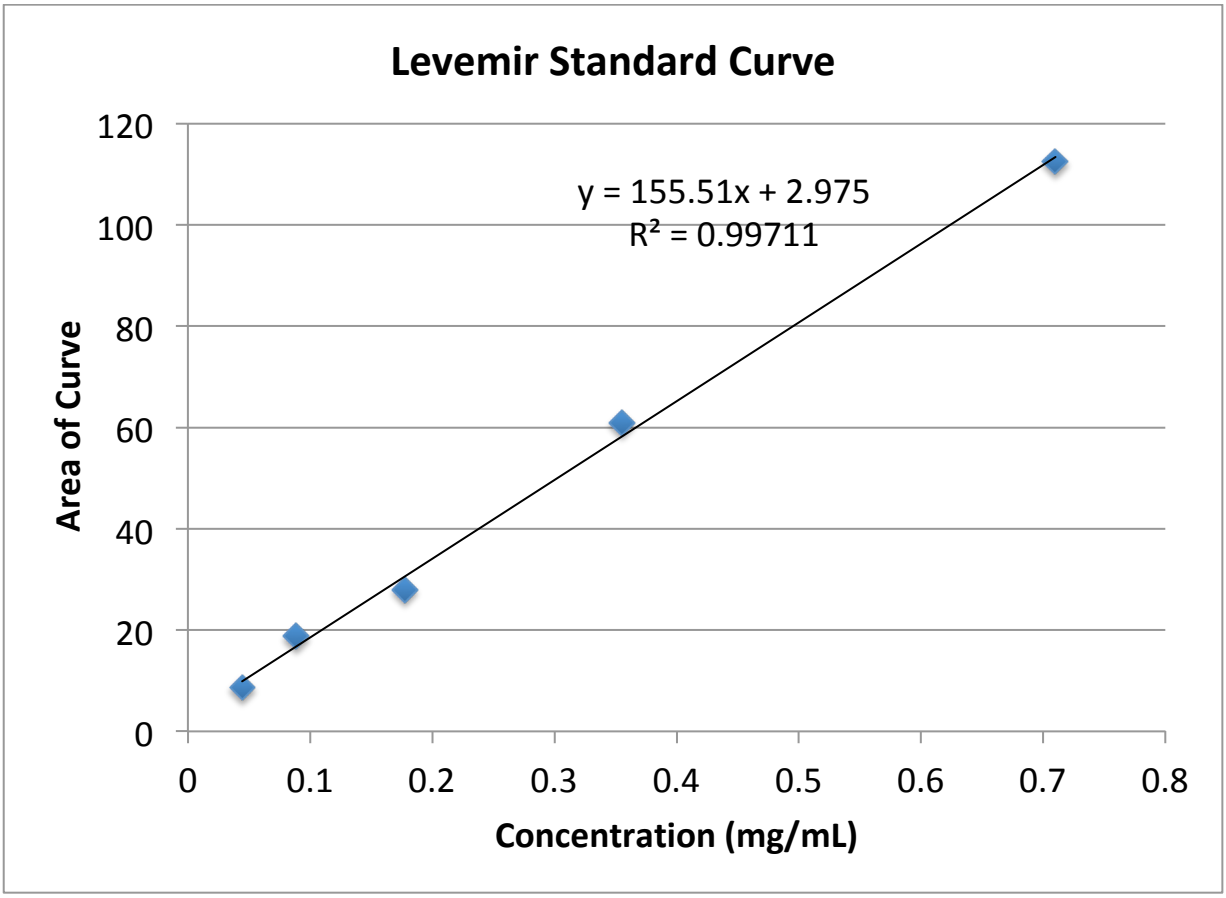

Figure 7: The standard curve will be used to find the incubated Levemir's concentration

From the three incubated samples, the average area is 65.7, which results in a concentration of 0.404 mg/mL of insulin. This is a 43.2% reduction in the insulin concentration after heating. The spectrophotometer reading's for the 20x heated Levemir and the 20x un-heated Levemir are shown in Figure 8.

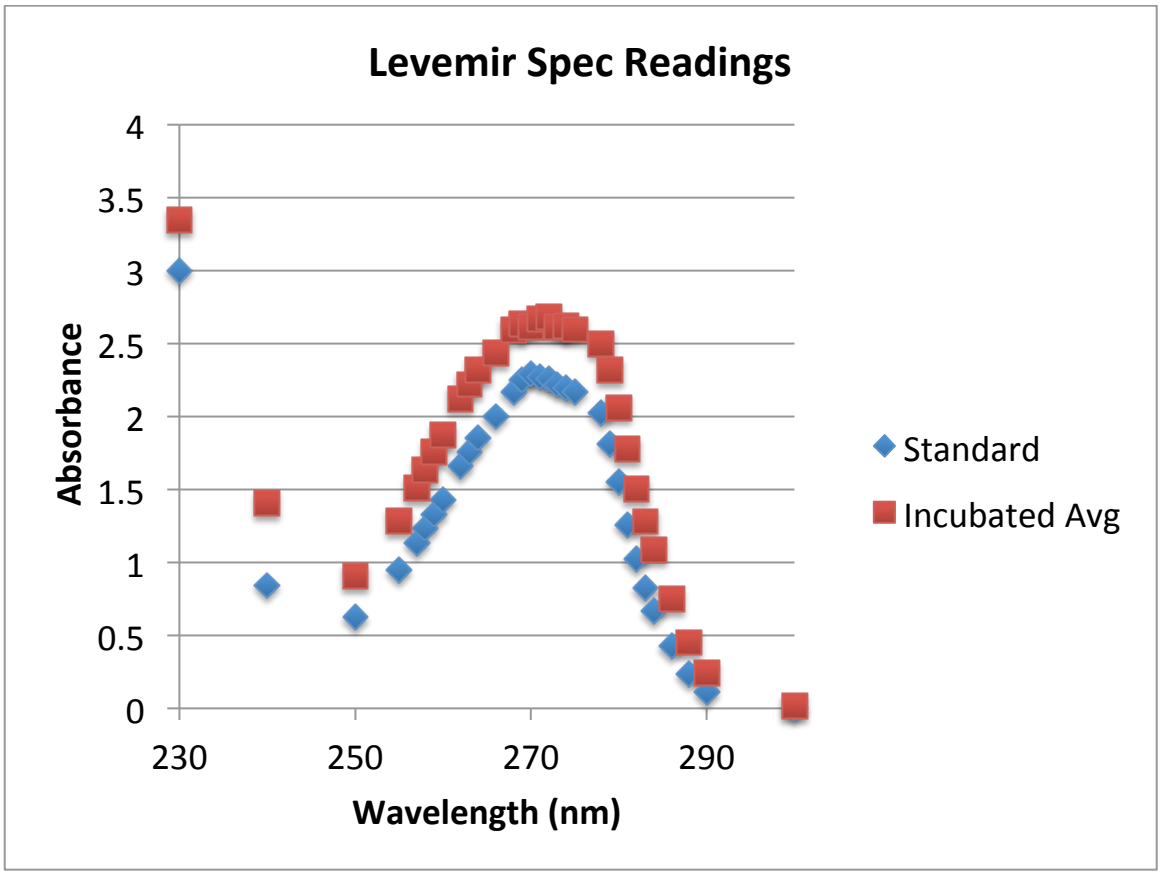

Figure 8: The incubated sample has a higher peak than the normal sample

4.4 Novolog:

The standard curve for the Novolog insulin is shown in Figure 9.

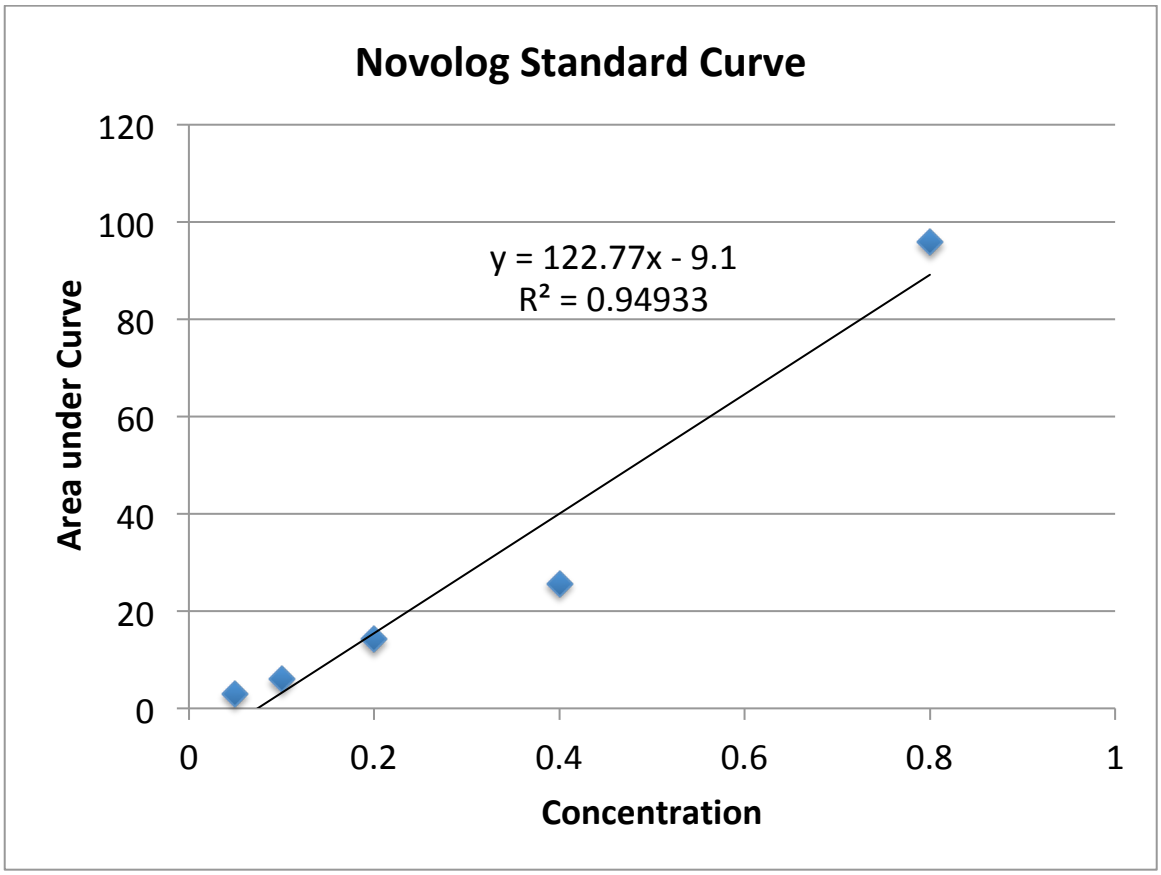

Figure 9: The standard curve will be used to find the incubated Levemir's concentration

From the three incubated samples, the average area is 38.6, which results in a concentration of 0.389 mg/mL of insulin. This is a 51.4% reduction in the insulin concentration after heating. The spectrophotometer reading's for the 20x heated Novolog and the 20x un-heated Novolog are shown in Figure 10.

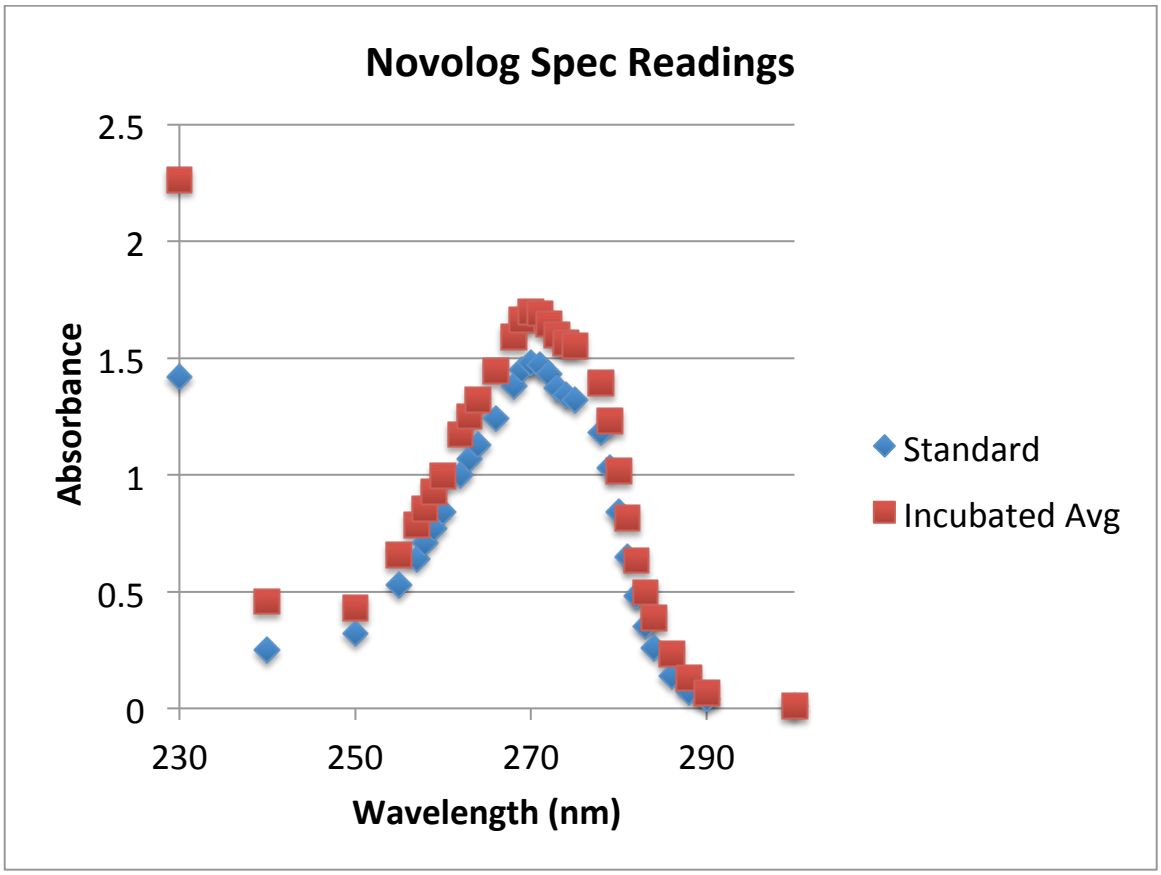

Figure 10: The incubated sample has a higher peak than the normal sample

5. Conclusions and Future Work:

The trend of a higher spectrophotometer peak at 270 nm and a lower insulin concentration was strongest for Levemir and Novolog. Humalog and Lantus had outliers in their spectrophotometer data that skewed the results lower. Therefore, it would be beneficial for future experiments to run more trials. The spike in absorbance in the spectrophotometer may have been due to oligopeptides resulting from the degrading insulin. These byproducts would not show up in the HPLC because all samples were filtered before entering the HPLC. Future experiments could also try exposing the insulin to light and extreme cold temperatures.

6. References

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[2] Mayer, J., F. Zhang and R. DiMarchi. "Insulin structure and function." Current Trends in Peptide Science (2007).

[3] Wen, L., et al. "Innate Immunity and Intestinal Microbiota in the Development of Type 1 Diabetes." Nature 455 (2008): 1109-1113.

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[5] Bekard, I. and D. Dunstan. "Tyrosine Autofluorescence as a Measure of Bovine Insulin Fibrillation." Biophys J. 97.9 (2009): 2521-2531.

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